Actin bundling protein fascin expression in ovarian neoplasms: Comparison of histopathologic features of tumors obtained by the first and secondary cytoreduction surgeries

S. Kabukcuoglu[1], *M.D., Asso. Prof.*; **S.S. Ozalp**[2], *M.D., Prof.*; **U. Oner**[1], *M.D., Prof.*; **K. Bildirici**[1], *M.D., Assist. Prof.*; **O.T. Yalcin**[2], *M.D., Prof.*; **T. Oge**[2], *M.D., Resident*; **E. Colak**[3], *PhD., Assist. Prof.*

Departments of Pathology[1], Gynecology and Obstetrics[2] and Biostatistics[3], University of Eskisehir Osmangazi School of Medicine, Eskisehir (Turkey)

Summary

Purpose of investigation: The aim of the study was to compare the fascin expression pattern and histopathologic features of malign epithelial ovarian tumors obtained by the primary and secondary surgeries.

Methods: The samples of 94 epithelial ovarian carcinomas, 35 secondary surgeries for ovarian carcinomas, 13 borderline epithelial ovarian tumors, 25 cystadenomas and four normal ovarian tissues were stained by means of fascin immunohistochemistry. Secondary surgeries included in the study were secondary cytoreduction at the time of second-look laparotomy (SLL), interval debulking surgery after neoadjuvant chemotherapy or secondary cytoreductive surgery in patients with recurrent epithelial ovarian carcinoma.

Results: Mean rank value of the stromal fascin score was higher in 94 cases of malign epithelial ovarian carcinomas than borderline epithelial tumors, cystadenomas and normal ovaries (.000, $p < 0.001$). There was no significant difference in terms of total epithelial fascin score (.685, $p > 0.05$) and total stromal fascin score (.572, $p > 0.05$) between the primary and the secondary surgeries of epithelial ovarian carcinomas.

Conclusions: Regarding the results of stromal fascin expression in 94 epithelial ovarian carcinomas, we hypothesized that cell-matrix interaction was an important step in the progression of malign epithelial ovarian neoplasms. Our study showed that the initial tumorigenic phenotype did not change with time and use of cisplatinum-based combination chemotherapy. Further studies with close follow-up of patients are necessary to reveal the role of fascin on matrix degradation mechanisms which might be the cause of the recurrences in ovarian neoplasms.

Key words: Epithelial ovarian carcinoma; Fascin immunohistochemistry; Second-look laparotomy; Tumorigenic phenotype.

Introduction

Epithelial ovarian carcinomas account for 80% to 90% of all ovarian malignancies. Many patients with epithelial ovarian carcinoma have been diagnosed with advanced stage of the disease [1]. Primary cytoreduction of ovarian carcinoma has been recomended for almost 30 years [2]. In advanced disease, surgery establishes the diagnosis, allows appropriate staging and cytoreduction. Survival has improved in patients with advanced stage ovarian cancer after aggressive cytoreduction followed by combination of platinum-based chemotherapy [3]. Secondary surgeries, which include secondary cytoreduction at the time of second-look laparotomy (SLL), interval debulking surgery after neoadjuvant chemotherapy or secondary cytoreductive surgery in patients with recurrent epithelial ovarian carcinoma, may be offered to patients with advanced stage of disease. The theory is that removal of large tumor nodules allows better penetration of chemotherapeutic agents and removes potential foci of chemoresistance [1-3]. Presence of stromal invasion and invasive implants are the most important histopathologic criteria in the diagnosis of aggressive ovarian neoplasms [4]. Recently, the role of matrix proteinases and tissue metalloprotease inhibitors in ovarian carcinoma progression has been investigated. The prognostic importance of matrix proteinases and extracellular matrix proteinase inducer (EMMPRIN), which is secreted by the microenvironment of the tumor cells and surrounding fibroblasts, has been demonstrated in ovarian carcinoma [5-7]. EMMPRIN, a member of the immunoglobulin superfamily of adhesion molecules with a molecular weight of 58kDa protein, is co-expressed with other metastasis-associated molecules in ovarian malignancy [6].

Actin filaments and actin bundling proteins are directly involved with cell motion, cell shape and nuclear polarization and cell-matrix interactions. Actin regulatory proteins provide mechanical support to cellular protrusions and stress fibers. Fascin, one of the actin regulatory proteins with a molecular weight of 55-58 kDa, has been expressed at high levels in specialized normal cells such

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as neuronal, endothelial and antigen presenting dendritic cells and many transformed cells [8-11]. The role of actin bundling protein fascin in tumor progression has been shown in malignant epithelial tumors including ovarian carcinoma [12]. In studies, fascin expression in breast cancer has been restricted to high-grade tumors. The up-regulation of fascin in estrogen and progesterone receptor-negative breast cancers implies that fascin may have a fundamental role in the acquisition of malign tumorigenic phenotype [13, 14].

In this study, our objectives were to investigate the fascin expression pattern of tumoral tissues which were obtained from the first and secondary cytoreduction surgeries of malign epithelial ovarian carcinomas and to make a comparison among fascin expression patterns of malign, borderline tumors, cystadenomas and normal ovaries.

Materials and Methods

Patients with 94 epithelial ovarian carcinomas, 13 borderline epithelial ovarian tumors, 25 cystadenomas and four normal ovaries were included in the study. All patients were surgically staged according to International Federation of Gynaecological Obstetrics (FIGO) criteria. Patient characteristics and clinicopathologic findings were obtained from hospital records and were retrived from the files at the Pathology Department between 1990 and 2000. Among 94 malign epithelial neoplasms, 35 cases that had had secondary cytoreduction surgery performed were selected for the study. Of the 35 secondary surgeries, 23 were SLL, two were interval debulking surgery after neoadjuvant chemotherapy and 11 were secondary cytoreductive surgery in patients with reccurent epithelial ovarian carcinoma. Optimal primary cytoreduction was defined as residual disease < 1 cm. Of the 23 patients who had SLL 14 had optimal cytoreductive surgery. Tumoral tissues which were obtained with the primary (n = 94) and secondary surgeries (n = 35) were microscopically reevaluated for grade, presence or absence of vascular invasion, calcification and degree of local cellular immune response. Paraffin sections which were selected from blocks of the most representative areas of invasive tumor were included in the study. The paraffin blocks were cut into 4 μm sections and immunohistochemical assays were performed for the expression of fascin using liquid mouse monoclonal antihuman fascin antibody (Novocastra, NCL-L-FASCIN, USA). Tissue sections were deparaffinized in xylene, rehydrated in alcohol solutions, and placed in 0.5% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase activity. Rehydration was completed by placing them in absolute alcohol and finally in water. The slides were treated with a boiling solution of freshly prepared 0.05 M-citrate buffer, pH 6.0 for 5 min in a pressure cooker. The sections were reacted overnight with the primary antibodies at a dilution of 1:200 in buffer. They were rinsed in phosphate buffered saline (PBS) before being treated with biotinylated universal secondary antibody for 10 min. After further rinsing, the slides were treated with avidin-biotin-peroxidase complex (Novocastra, Novostain Universal Detection Kit) and rinsed again. Immunostaining was accomplished by incubating them with 3amino-α-ethylcarbazole (AEC) for 7 min and then the slides were rinsed in distilled water and counter-stained with Mayer's hematoxylin. Sections of human tonsil were used as positive controls. Capillary endotheliums were also used as endogenous positive controls. As a negative control, the primary antibody was replaced by PBS.

The epithelial fascin score for each tumor specimen was the sum of the percentage score and the intensity score and was defined as 'total epithelial fascin score'. Tissue samples were divided into three groups according to percentage of stained cells; ≤ 10% as 1, 11-50% as 2 and 51-100% as 3. The intensity of immunostaining was scored on a three-point scale: 1 = weak; 2 = moderate; 3 = intense. Stromal staining was scored on a three point scale: focal weak staining as 1; regional moderate staining as 2; diffuse and moderate to intense staining as 3.

All statistical analyses were performed using SPSS (Statistical Package of Social Services, Chicago, IL, USA) for Windows version 11.5. Data were analyzed according to the Kruskal-Wallis test, Wilcoxon signed ranks test, McNemar test, Pearson chi-square test and the Exact Pearson chi square test. Probability values less than 0.05 were considered statistically significant.

Results

Mean ages of patients with normal ovaries, cystadenomas, borderline ovarian neoplasms, malign epithelial ovarian neoplasms and the secondary surgery group were 46, 47, 35, 55 and 52, respectively. The length of follow-up ranged from six months to 94 (mean = 34.45 ± 21.83) months. Of the 35 patients in the second group, ten patients died of disease and six patients were alive with disease. Of the 94 malign epithelial ovarian carcinoma patients, 18 were early (Stage I-II) and 76 were late (Stage III-IV) stages. Of the 35 secondary surgery group the initial stage was early in two patients and late in 33 patients. Table 1 shows the distribution of stages, and the clinical and histopathological features of biopsies after pathologic review.

Table 1. — *Stages, grade and histologic subtype of malign epithelial ovarian neoplasms.*

Variable	Carcinoma No. = 94	Carcinoma with secondary surgery No. = 35	Borderline tumors No. = 13
Stage			
IA	6		7
IB	4		3
IC	4	2	2
IIA	2		
IIC	2		
IIIA	5	1	
IIIB	1		1
IIIC	59	29	
IV	11	1	
Unstaged		2	
Grade			
1	23	9	
2	58	23	
3	13	3	
Histologic subtype			
Serous	64	26	4
Endometrioid	15	5	1
Clear	4		
Mucinous	4	3	8
Mixed	5		
Transisitonal	2	1	

Intensity of local cellular immune response was weak in 17 (65%) cystadenomas, in 11 (84%) borderline ovarian tumors, in 57 (60%) ovarian carcinomas and in 25 (71%) samples of secondary surgeries. Moderate to intense local cellular immune response was observed in one (4%) cystadenoma, in two (15%) borderline ovarian tumors, in 35 (37%) malign ovarian tumors and in nine (25%) samples of secondary surgeries.

Vascular invasion was observed in 81 (86%) of 94 malign epithelial ovarian tumors and in 32 (90%) of 35 samples of secondary surgeries. Psammomatous calcifications were observed in three (12%) of 25 cystadenomas, in three (25%) of 13 borderline tumors, in 43 (45%) of 94 malign ovarian tumors and in 25 (71%) of 35 samples of secondary surgeries. Table 2 shows a comparison of histopathologic features of the two groups with benign and borderline ovarian tumors.

Presence of psammomatous calcifications ($p = 0.001$), local cellular immune response (.000, $p < 0.001$) and vascular invasion (.000, $p < 0.001$) were significantly high in the first group of malign neoplasms. The same results were also obtained in the group of secondary surgeries and p values were .000 for three parameters.

Fascin immunohistochemistry results

Normal ovarian tissue

Moderate fascin staining in normal ovarium was marked in every developmental stage of follicular epithelium and corpus luteum. Cortical stromal tissue was stained with fascin in focal areas.

Cystadenoma

Epithelium of mucinous cystadenomas were not stained with fascin. Stromal fibroblasts were stained weak to moderate intensity in focal areas in all samples. Weak epithelial fascin staining score ranging from 2 to 3 was observed in five of 11 serous cystadenomas. Stromal fibroblasts were stained weak to intense in ten of 11 samples.

Borderline epithelial ovarian neoplasms

In mucinous borderline tumors the total epithelial fascin score was 2 in five of eight samples. Fascin scores of stromal fibroblasts ranged from 1 to 3 in all samples. Epithelium of serous borderline tumors were stained with fascin in three of four samples with the scores of 2 to 6. Fascin scores of stromal fibroblast ranged from 1 to 3 in four samples. Epithelium and stromal fibroblasts stained weakly in one sample of endometrioid borderline tumor.

Malignant ovarian carcinoma

Fascin staining in stroma was observed in 92 (97%) of 94 samples. The stromal fascin scores ranged from 1 to 3. The epithelial portion of tumor was stained with fascin in 61 (64%) of 94 samples. The epithelial fascin scores ranged from 2 to 5. Serous carcinomas containing dense psammomatous calsifications did not show any epithelial or stromal fascin staining. The areas of squamous metaplasia in endometrioid carcinomas showed intense fascin labelling.

Comparison of ovarian carcinoma tissues obtained with the primary and secondary surgeries

The stromal fascin scores ranged from 1 to 3 for both groups. The epithelial fascin scores ranged from 2 to 5 in the primary surgeries and 2 to 6 in the secondary surgeries (Figures 1 and 2). Negative epithelial fascin staining was observed in 11 samples whereas positive staining was observed in the tumoral tissues obtained by the primary or secondary surgeries. Tables 3 and 4 show a comparison of results in fascin staining in the primary and secondary surgeries.

There was no significant difference in terms of epithelial fascin scores in the benign group, borderline tumors and malign ovarian tumors which were obtained from the primary surgeries ($p = .080$, $p > 0.05$) and the secondary surgeries ($p = .052$, $p > 0.05$). Stromal fascin scores of invasive (94 cases) and borderline epithelial ovarian tumors were significantly higher than for the benign group (.000, $p < 0.001$). The same test was not significant for the secondary surgeries of invasive carcinoma (.331, $p > 0.05$). Table 3 shows a comparison of fascin immunoreactivity. The epithelial fascin scores (.685, $p > 0.05$) and stromal fascin scores (.572, $p > 0.05$) of the primary and secondary surgeries were not significantly

Table 2. — *Comparison of histopathologic features of two groups with benign, borderline ovarian neoplasms.*

Groups	Primary surgeries No. = 94				p	Secondary surgeries No. = 35				p
Calcification		Benign	Borderline	Malign	.001*		Benign	Borderline	Malign	.000*
	–	26	10	51		–	26	10	10	
	+	3	3	43		–	3	3	25	
		29	13	94			29	13	35	
Local cellular	0	11	0	2	.000*	0	11	0	1	.000*
immune	1	17	11	57		1	17	11	25	
response	2	1	2	35		2	1	2	9	
		29	13	94			29	13	35	
Lymphovascular	–	29	12	14	.000*	–	29	12	4	.000*
space	+	0	1	80		+	0	1	31	
involvement		29	13	94			29	13	35	

*Statistically important significance (chi square tests).

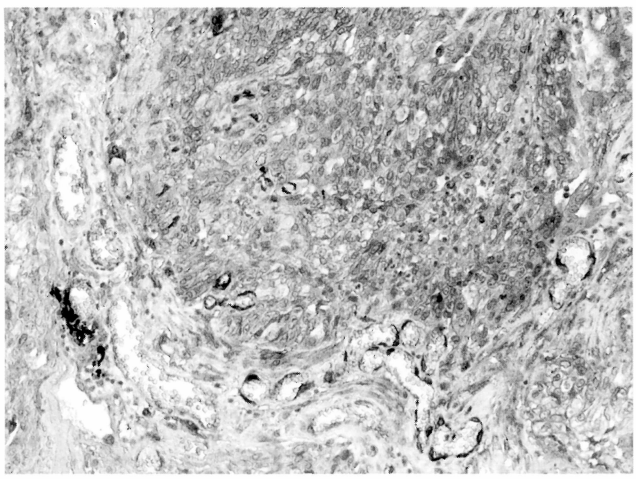

Fig. 1

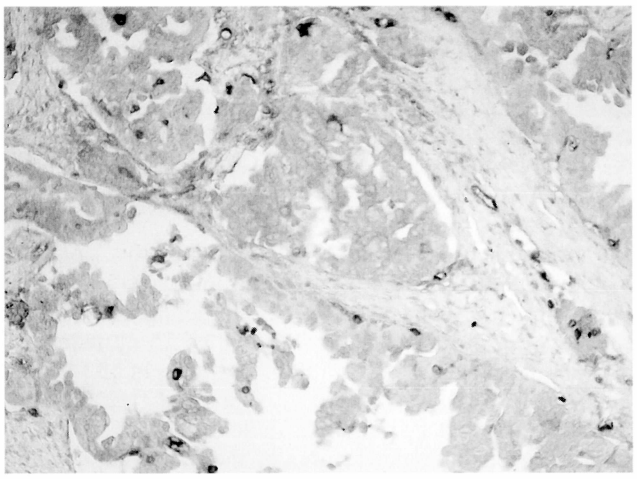

Fig. 2

Figure 1. — Primary surgery for serous carcinoma. Optimal cytoreduction was performed in this case. Fascin expression of tumor cells, stroma and microvessel endothelium (x 400).
Figure 2. — Second-look surgery of the same patient. The patient was lost to follow-up after 16 months (x 400, fascin immunohistochemical staining).

Table 3. — *Comparison of stromal fascin scores and total epithelial fascin scores of the primary and secondary surgery groups, benign and borderline ovarian neoplasms.*

Stromal fascin score range: 0-3	Group	No.	Mean rank	.000**	Group	No.	Mean rank	.331	Kruskal-Wallis
	Benign*	29	39.81		Benign*	29	35.38		
	Borderline	13	51.38		Borderline	13	36.92		
	Malign	94	79.81		Malign	35	42.77		
Total epithelial fascin score range: 0-6	Group	No.	Mean rank	.080	Group	No.	Mean rank	.052	Kruskal-Wallis
	Benign*	29	54.84		Benign*	29	31.74		
	Borderline	13	67.81		Borderline	13	39.88		
	Malign	94	72.81		Malign	35	44.69		

*The benign group consisted of 25 cystadenoma samples and 4 normal ovarian tissue samples.
**Statistically important significance (chi square tests).

different. A comparison of the results of fascin staining in the primary and secondary surgeries is shown in Table 4 and Table 5. Presence or absence of lymphatic vessel invasion was not associated with disease-free survival (.206, $p > 0.05$) and overall survival (.646, $p > 0.05$). There was no significant correlation between the stage and epithelial fascin scores ($r = .078$, $p = .462$), stage and disease-free survival ($r = -.225$, $p = .067$) and overall survival ($r = -.195$, $p = .321$) in 94 cases of malign ovarian tumors.

Table 4. — *Comparison of epithelial fascin immunoreactivities in the primary and secondary cytoreductive surgeries.*

	Ranks				p
Epithelial fascin score		No.	Mean rank	Sum of ranks	.685*
	Negative ranks	13	10.46	136	
	Positive ranks	11	14.91	164	
	Ties	11			

*Wilcoxon signed ranks test.

Table 5. — *Comparison of stromal fascin immunoreactivities in the primary and secondary surgeries.*

Score					p
	.00	1.00	2.00	3.00	.572*
.00	0	1	0	0	
1.00	3	1	5	5	
2.00	0	5	4	5	
3.00	1	1	2	2	

*McNemar test.

Discussion

In this study, we investigated the change of initial tumorigenic phenotype in malign epithelial ovarian neoplasms with the time or reccurence of the tumor by using fascin immunohistochemistry and histopathologic parameters. The role of fascin on expression in generating and maintaining dynamic tumorigenic phenotype has been supported in several types of human neoplasms including some lymphoproliferative disorders, Hodgkin's lymphoma, breast, ovary, and pancreas carcinomas, small and non-small cell lung carcinomas, osephageal, gastric and colonic carcinomas, and skin tumors [15-24]. Fascin shows strong positivity in all cases of classic Hodgkin disease [16]. Down-regulation of fascin and loss of cell-cell, cell-matrix adhesions also have an important role in malignant tumor progression in some tumors, such as

melanomas [24-26]. In this study we observed various degrees of epithelial fascin staining in 20% of cystadenomas, in 62% of borderline epithelial tumors and in 64% of invasive epithelial ovarian tumors. The stromal fascin scores of invasive (94 cases) and borderline epithelial ovarian tumors were significantly higher compared to the benign group (.000, $p < 0.001$). Hu *et al.* [12] demonstrated increased fascin expression in tissue samples and cell cultures derived from ovarian cancer and in tissues of borderline and carcinomatous ovarian neoplasms, and suggested that fascin could serve as an important prognostic factor for abnormal ovarian epithelial pathology.

Comparison of malignant epithelial ovarian neoplasms with cystadenomas and normal ovaries demonstrated that the scores of stromal fascin expression ($p < .001$), presence or absence of local cellular immune response ($p < .001$), vascular invasion ($p < .001$) and psammomatous calcifications ($p = .001$) were significantly higher in malignant epithelial ovarian neoplasms. The same results were also obtained in the group of secondary surgeries and p values were .000 for each parameter. Tumor invasion and metastasis are controlled by degradation of the extracellular matrix components. Presence of stromal invasion and invasive implants are the most important histopathologic criteria in the diagnosis of invasive epithelial ovarian neoplasms [4, 5]. In our study we observed that presence or absence of lymphatic vessel invasion was not associated with disease-free survival and overall survival. There were no significant correlations between stage and epithelial fascin scores, stage and disease-free survival and overall survival. These results showed that epithelial and stromal fascin staining, lymphovascular space involvement, local cellular immune response and cell-matrix interaction were associated with aggresive tumorigenic phenotype, and initial tumorigenic phenotype did not change significantly with the time and after cisplatin-based combined chemotherapy.

Shonukan *et al.* [27] demonstrated that there were high levels of neurotrophin expression in the normal tissue adjacent to brain metastases of melanoma. It was suggested that interaction between fascin and neurotrophin provides a direct link between the NGF signaling pathway and neurotrophin-mediated melanoma cell movement by down-regulation (dephosphorilation) of fascin. The TNF/NGF pathway is associated with alterations in the regulation of apoptosis and resistance of tumor cells to therapy [28-35]. These studies and our data suggested that both up-regulation and down-regulation of fascin in tumoral tissue may promote invasion of ovarian carcinoma by cell-matrix adhesion and may have a role in the acquisition of chemoresistance.

Our study demonstrates the importance of actin bundling protein fascin in the progression of ovarian neoplasms. Further studies with close follow-up of patients are necessary to reveal the role of fascin on matrix degradation mechanisms which might be the cause of recurrence of ovarian neoplasms.

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Address reprint requests to:
S. KABUKCUOGLU, M.D.
Visnelik Mah. Taskopru cad.
Yalcin Sitesi,
B-Blok, D-13,
26020 Eskisehir (Turkey)